

CLAIMS

1. A method comprising:

a) providing:

i) a first recombinant vector, comprising in operable combination:

- 1) a nucleotide sequence of interest having a 5' end and a 3' end;
- 2) left and right inverted terminal repeats of adenovirus flanking said nucleotide sequence of interest;
- 3) adenovirus packaging sequence linked to one of said inverted terminal repeats; and
- 4) an adeno-associated virus terminal repeat sequence operably linked to said 3' end of said nucleotide sequence of interest,

wherein said first vector lacks a second adeno-associated virus terminal repeat sequence, and lacks one or more adenovirus early gene region selected from E1, E2, E3, and E4 gene region; and

ii) a cell capable of expressing said one or more adenovirus early gene which is lacking from said first vector;

b) introducing said first vector into said cell to produce a transformed cell; and

c) culturing said transformed cell under conditions such that a second vector is produced, said second vector selected from:

- i) a third vector, comprising in operable combination:
 - 1) adeno-associated virus terminal repeat DD sequence;

2) first and second inverted copies of a nucleotide sequence of interest flanking said adeno-associated virus terminal repeat-DD sequence;

3) left and right inverted terminal repeats of adenovirus flanking said first and second inverted copies of said nucleotide sequence of interest; and

4) an adenovirus packaging sequence linked to one of said inverted terminal repeats, and

ii) a fourth vector, comprising in operable combination:

1) a nucleotide sequence of interest having a 5' end and a 3' end;

2) left and right inverted terminal repeats of adenovirus flanking said nucleotide sequence of interest; and

3) an adenovirus packaging sequence linked to one of said inverted terminal repeats.

2. The method of Claim 1, wherein said cell is capable of expressing one or more Rep proteins, and said culturing results in expression of said one or more Rep proteins.

3. The method of Claim 1, wherein said second vector is encapsidated.

4. The method of Claim 3, further comprising d) recovering said encapsidated second vector.

5. The method of Claim 4, further comprising e) purifying said recovered encapsidated second vector.

6. The method of Claim 5, further comprising e) administering said purified encapsidated second vector to a host cell.

7. The method of Claim 6, wherein said administering is under conditions such that said nucleotide sequence of interest in said encapsidated second vector is expressed.

8. The method of Claim 6, wherein said host cell is a cultured cell.

9. The method of Claim 6, wherein said host cell is comprised in a mammal.

10. The method of Claim 9, wherein said mammal is selected from mouse and human.

11. The method of Claim 2, wherein expression of one or more Rep proteins is inducible.

12. A method comprising:

a) providing:

i) a first recombinant vector, comprising in operable combination:

1) a nucleotide sequence of interest having a 5' end and a 3' end;

2) left and right inverted terminal repeats of adenovirus flanking said nucleotide sequence of interest;

3) adenovirus packaging sequence linked to one of said inverted terminal repeats; and

- 4) an adeno-associated virus terminal repeat sequence operably linked to said 3' end of said nucleotide sequence of interest,

wherein said first vector lacks a second adeno-associated virus terminal repeat sequence, and lacks one or more adenovirus early gene region selected from E1, E2, and E4 gene region;

- ii) a cell capable of expressing one or more Rep proteins; and

- iii) helper adenovirus;

b) introducing said first vector and genome of said helper adenovirus into said cell to produce a transformed cell; and

c) culturing said transformed cell under conditions such that said transformed cell expresses said one or more Rep proteins, and a second vector is produced, said second vector selected from:

- i) a third vector, comprising in operable combination:

- 1) adeno-associated virus terminal repeat DD sequence;

- 2) first and second inverted copies of a nucleotide sequence of interest flanking said adeno-associated virus terminal repeat-DD sequence;

- 3) left and right inverted terminal repeats of adenovirus flanking said first and second inverted copies of said nucleotide sequence of interest; and

- 4) an adenovirus packaging sequence linked to one of said inverted terminal repeats, and

- ii) a fourth vector, comprising in operable combination:

- 1) a nucleotide sequence of interest having a 5' end and a 3' end;

- 2) left and right inverted terminal repeats of adenovirus flanking said nucleotide sequence of interest; and
- 3) an adenovirus packaging sequence linked to one of said inverted terminal repeats

13. The method of Claim 12, wherein said cell lacks expression of said one or more adenovirus early gene region which is lacking from said first vector.

14. A method comprising:

a) providing:

i) a first recombinant vector, comprising in operable combination:

- 1) a nucleotide sequence of interest having a 5' end and a 3' end;
- 2) left and right inverted terminal repeats of adenovirus flanking said nucleotide sequence of interest;
- 3) adenovirus packaging sequence linked to one of said inverted terminal repeats; and
- 4) an adeno-associated virus terminal repeat sequence operably linked to said 3' end of said nucleotide sequence of interest,

wherein said first vector lacks a second adeno-associated virus terminal repeat sequence, and lacks one or more adenovirus early gene region selected from E1, E2, and E4 gene region;

- ii) a cell capable of expressing said one or more adenovirus early gene which is lacking from said first vector; and
- iii) adeno-associated virus;

- b) introducing said first vector and genome of said adeno-associated virus into said cell to produce a transformed cell; and
- c) culturing said transformed cell under conditions such that a second vector is produced, said second vector selected from:

i) a third vector, comprising in operable combination:

1) adeno-associated virus terminal repeat DD sequence;

2) first and second inverted copies of a nucleotide sequence of interest flanking said adeno-associated virus terminal repeat-DD sequence;

3) left and right inverted terminal repeats of adenovirus flanking said first and second inverted copies of said nucleotide sequence of interest; and

4) an adenovirus packaging sequence linked to one of said inverted terminal repeats, and

ii) a fourth vector, comprising in operable combination:

1) a nucleotide sequence of interest having a 5' end and a 3' end;

2) left and right inverted terminal repeats of adenovirus flanking said nucleotide sequence of interest; and

3) an adenovirus packaging sequence linked to one of said inverted terminal repeats.

15. A method comprising:

a) providing:

i) a first recombinant vector, comprising in operable combination:

- 1) a nucleotide sequence of interest having a 5' end and a 3' end;
- 2) left and right inverted terminal repeats of adenovirus flanking said nucleotide sequence of interest;
- 3) adenovirus packaging sequence linked to one of said inverted terminal repeats; and
- 4) an adeno-associated virus terminal repeat sequence operably linked to said 3' end of said nucleotide sequence of interest,

wherein said first vector lacks a second adeno-associated virus terminal repeat sequence, and lacks adenovirus E3 early gene region; and

- ii) a cell;
- b) introducing said first vector into said cell to produce a transformed cell; and
- c) culturing said transformed cell under conditions such that a second vector is produced, said second vector selected from:
 - i) a third vector, comprising in operable combination:
 - 1) adeno-associated virus terminal repeat DD sequence;
 - 2) first and second inverted copies of a nucleotide sequence of interest flanking said adeno-associated virus terminal repeat-DD sequence;
 - 3) left and right inverted terminal repeats of adenovirus flanking said first and second inverted copies of said nucleotide sequence of interest; and
 - 4) an adenovirus packaging sequence linked to one of said inverted terminal repeats, and
 - ii) a fourth vector, comprising in operable combination:

- 1) a nucleotide sequence of interest having a 5' end and a 3' end;
- 2) left and right inverted terminal repeats of adenovirus flanking said nucleotide sequence of interest; and
- 3) an adenovirus packaging sequence linked to one of said inverted terminal repeats.

16. The method of Claim 15, wherein said cell is capable of expressing one or more Rep proteins, and said culturing results in expression of said one or more Rep proteins.

17. A method comprising:

- a) providing:
 - i) a first recombinant vector, comprising in operable combination:
 - 1) a nucleotide sequence of interest having a 5' end and a 3' end;
 - 2) left and right inverted terminal repeats of adenovirus flanking said nucleotide sequence of interest;
 - 3) adenovirus packaging sequence linked to one of said inverted terminal repeats; and
 - 4) an adeno-associated virus terminal repeat sequence operably linked to said 3' end of said nucleotide sequence of interest,

wherein said first vector lacks a second adeno-associated virus terminal repeat sequence, and wherein said nucleotide sequence of interest in said first vector comprises adeno-associated virus rep gene region; and

- 5
- ii) a cell;
- b) introducing said first vector into said cell to produce a transformed cell; and
- c) culturing said transformed cell under conditions such that said transformed cell expresses one or more Rep proteins, and a second vector is produced, said second vector selected from:
- 10
- i) a third vector, comprising in operable combination:
- 1) adeno-associated virus terminal repeat DD sequence;
- 2) first and second inverted copies of a nucleotide sequence of interest flanking said adeno-associated virus terminal repeat-DD sequence;
- 15
- 3) left and right inverted terminal repeats of adenovirus flanking said first and second inverted copies of said nucleotide sequence of interest; and
- 4) an adenovirus packaging sequence linked to one of said inverted terminal repeats, and
- 20
- ii) a fourth vector, comprising in operable combination:
- 1) a nucleotide sequence of interest having a 5' end and a 3' end;
- 2) left and right inverted terminal repeats of adenovirus flanking said nucleotide sequence of interest; and
- 25
- 3) an adenovirus packaging sequence linked to one of said inverted terminal repeats.

18. The method of Claim 17, wherein said first vector lacks one or more adenovirus early gene region selected from E1, E2, and E4 gene region, and said cell

is capable of expressing said adenovirus early gene region which is lacking from said first vector.

19. The method of Claim 17, wherein said first vector lacks adenovirus E3 gene region.

5

10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100
105
110
115
120
125
130
135
140
145
150
155
160
165
170
175
180
185
190
195
200
205
210
215
220
225
230
235
240
245
250
255
260
265
270
275
280
285
290
295
300
305
310
315
320
325
330
335
340
345
350
355
360
365
370
375
380
385
390
395
400
405
410
415
420
425
430
435
440
445
450
455
460
465
470
475
480
485
490
495
500
505
510
515
520
525
530
535
540
545
550
555
560
565
570
575
580
585
590
595
600
605
610
615
620
625
630
635
640
645
650
655
660
665
670
675
680
685
690
695
700
705
710
715
720
725
730
735
740
745
750
755
760
765
770
775
780
785
790
795
800
805
810
815
820
825
830
835
840
845
850
855
860
865
870
875
880
885
890
895
900
905
910
915
920
925
930
935
940
945
950
955
960
965
970
975
980
985
990
995